REVISTA ESPAÑOLA DE FISIOLOGIA, 41, 145-150. 1985

Transport of Calcium, Magnesium and Strontium by Human Serum Proteins

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(Received on June 14, 1984)

J. O. ALDA and J. F. ESCANERO. *Transport of Calcium, Magnesium and Strontium by Human Serum Proteins.* Rev. esp. Fisiol., 41, 145-150. 1985.

The association constant and the maximal binding capacity for calcium, magnesium and strontium to human serum proteins were determined using a wide concentration range by a dialysis technique. The association curves are similar for these elements. Each curve may be subdivided in three parts: the first would correspond to the sub-fraction tightly bound to protein, the second one shows a linear design implying the association of the element to different binding points which have the same association constant, and the third part shows the saturation of proteins, which is achieved by alkaline-earth concentration about 9 times higher than the physiological ones.

Key words: Calcium, Carrier proteins, Magnesium, Strontium.

Three calcium plasmatic fractions are known: ionic calcium, calcium bound to small anions and calcium bound to proteins (6). These fractions are in chemical equilibrium (11), so that any changes in the ionized calcium concentrations will produce a proportional change in the protein bound calcium concentration. It is believed that calcium bound to proteins has a reservoir function of ionized calcium, while this ionized calcium fraction would be the active one subjected to homeostatic control (7).

This paper proposes to estimate the binding of alkaline earth ions —calcium, magnesium and strontium— to human serum proteins on wide range of concentrations also without the interference of other alkaline earth elements.

Materials and Methods

Serum from human donors, aged 18-25 years old, without hepatic, bone or renal disease was used to make 30 pools, 10 for each one of these elements: cal-

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cium, magnesium and strontium. Every sample was deprived of alkaline earth ions using a chromatographic technique previously described (1). The chromatographied sera were subdivided in eight aliquots containing 4 ml each, to which different concentrations of ions were added to make several final concentrations from 0.12 up to 55 mmol/l of Ca, from 0.025 up to 70 mmol/l of Mg and from 0.07 up to 6 mmol/l of Sr. Then each aliquot was subjected to dialysis according to the LADENSON and SHYONG technique (4) keeping temperature, pH and ionic strength within physiological levels.

Calcium, magnesium and strontium were determined by atomic absorption spectrophotometry. The total serum protein concentrations (TPr) were determined by biuret reaction. The obtained values were used to draw the association curves and establish the association constant (K_{PrX}) and the maximal binding capacity or number of binding sites per gramme of total proteins (n_X) for each element (x), according to McLEAN and HASTINGS (6). They proposed that alkaline earth metal ions bind to plasma proteins in accordance with the mass action law:

$$K_{PrX} = (PrX)/(X^{++})(Pr^{-}) \qquad [I]$$

where (Pr^{-}) is the total binding sites concentration available for X^{++} and is equal to:

$$(Pr^{-}) = n(TPr) - (PrX) \qquad [II]$$

and (PrX) is the divalent element bound to protein substituting [II] into [I] gives:

$$K_{PrX} = (PrX)/(X^{++})\,[n(TPr) - (PrX)]$$

rearranging:

$$K_{PrX} \cdot (X^{++}) = (PrX)/[n(TPr) - (PrX)]$$

$$1/K_{PrX} \cdot (X^{++}) =$$

$$= [n(TPr) - (PrX)]/(PrX) =$$

$$= [n \cdot (TPr)/(PrX)] - 1$$

finally:

$$TPr/PrX =$$

$$= (1/K_{PrX} \cdot n) \cdot (1/X^{++}) + (1/n) \qquad [III]$$

The last equation is a regression line that needs the values of ionic elements, but their determinations are not reliable at the moment for Sr and Mg. To solve the small anion interferences, the serum was deprived of phosphate, citrate and carbonate by chromatography with Dowex 1 chloride.

Results

Calcium association curves. — The results of different percentages of ultrafiltrable calcium found at the different concentrations of calcium in serum are shown in figure 1. On the right side are represented the results of calcium bound to proteins from 1 mmol/l interpreted according to equation [III].

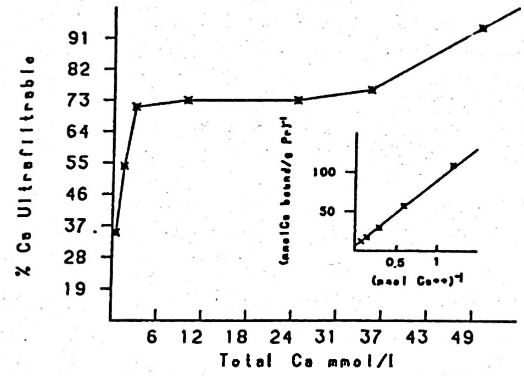

Fig. 1. *Calcium association curve: percentage of ultrafiltrable calcium versus total calcium in serum*

On the right (mmol of calcium bound/g of total protein)$^{-1}$ versus (mmol/l of Ca^{++})$^{-1}$ are represented.

The main graph may be subdivided in three parts in order to be correctly interpreted: the first one up to 1 mmol/l, the second one implies that the association is maintained constant up to 30 mmol/l, the third one shows a decrease of element bound when the concentrations are increased above this value.

In the right figure the regression line value at the origin of the ordinate is 5.12 that corresponds to maximal binding capacity of 0.19 mmol of Ca/g of proteins. The slope gives a value of 92 which represents a K_{PrCa} of 55.7 ± 18 (SD) M^{-1}.

Magnesium association curves. — The values of ultrafiltrable magnesium to different final concentrations of magnesium in serum are represented in figure 2 where three parts can be considered: the first corresponds to lower values than 0.024 mmol/l, the second one, up to concentration 30 mmol/l and the third one, beyond this level.

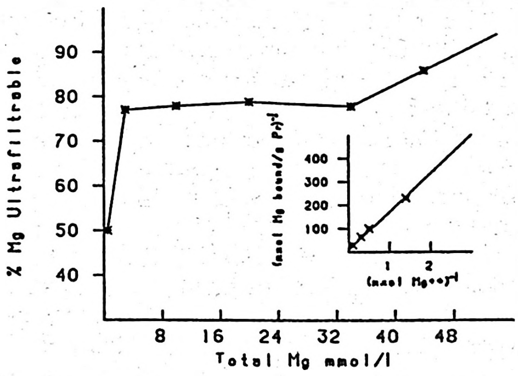

Fig. 2. *Magnesium association curve: percentage of ultrafiltrable magnesium versus total magnesium in serum.*
On the right (mmol of magnesium bound/g of total proteins)$^{-1}$ versus (mmol/l of Mg^{++})$^{-1}$ are represented.

In the figure at the right are represented the results of magnesium bound to proteins according to equation [III] from 0.5 mmol/l. The regression line value at the origin is 5.55 that corresponds to a maximal binding capacity of 0.18 mmol of Mg/g of proteins. The slope gives a value of 33.23 which represents an association constant of 167 ± 25 M^{-1}.

Strontium association curves. — The values of the association of this element are represented in figure 3 where three parts can be considered: the first has an upper limit of 0.05 mmol/l, the second up to 5.5 mmol/l and the third part starts from this level.

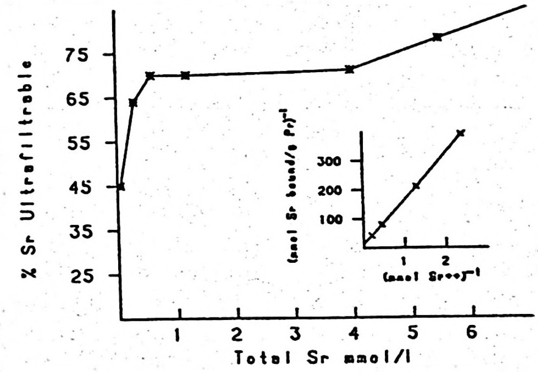

Fig. 3. *Strontium association curve: percentage of ultrafiltrable strontium versus total strontium in serum.*
On the right (mmol/l of strontium bound/g of total proteins)$^{-1}$ versus (mmol/l of Sr^{++})$^{-1}$ are represented.

At the right, the results of strontium bound to proteins from 1 mmol/l are represented according to equation [III]. The regression line values at the origin of the ordinate was of 7.76 that corresponds to a maximal binding capacity of 0.128 mmol of Sr/g of proteins. The slope gives a value of 156 which represents a K_{PrSr} of 49.9 ± 16 M^{-1}.

Discussion

The figures representing the elements bound to proteins have several similarities: all three increase their binding percentages with small concentrations; these percentages are quite constant at middle concentrations and all of them decrease at the highest concentrations.

Table I. *Association constants and maximal binding capacities between calcium and serum proteins reported by different authors.*

Serum	Method	(°C)	pH	n(mmol)	KprCa(M^{-1})	Ref.	Date
Human	Frog heart	22	—	7.8	166.0 ± 1.2	(6)	1935
Bovine	Centrifugation	37	7.35	6.3	151.0	(5)	1960
Rabbit	Ultrafiltration	22	7.35	7.8	60.2	(8)	1961
Bovine	Ultrafiltration	22	7.35	—	45.7- 48.9	(10)	1961
Rat	Dialysis	37	7.35	2.5	281.0 ± 2.0	(9)	1973
Human	Ultrafiltration	37	7.40	0.12*	124.0	(7)	1972
Human	Dialysis	37	7.40	0.19*	55.7 ± 18.0	this study	

* Values expressed as mmol/g of protein.

The plateau in the figures must be due to the presence of many binding points with a similar association constant. The second slope, in which the percentage of binding to proteins decreases, means total saturation of binding points. When the concentrations of the element added are small the binding increases. These data, interpreted from the binding model to different proteins (10), might be understood as tight binding, this could be explained by the presence of a smaller constant of association in some protein. These dialysis data for calcium agree with those reported by WORTSMAN and TRAYCOFF (12), who have already suggested the presence of a Ca fraction tightly bound to proteins, adding moreover that this subfraction is also present for Mg and Sr. However, we have found these results only by dialysis (5) but not by ultrafiltration (2); consequently the fraction tightly bound to proteins must be confirmed since our results differ according to the technique used.

The mean value calculated for the association constant for the calcium fraction not tightly bound is lower than that reported by PEDERSEN (7) and MAC LEAN and HASTING (6) (table I) for human serum; this must be the effect of different techniques used of the widened concentration range we used. The data of magnesium are scarce; FRYE *et al.* (3), working with bovine albumin, calculated an association constant of 92.6 M. Data were not found for the Sr association constant.

The results of the maximal binding capacity for calcium reported by other authors expressed in mmol/l can be seen in table I. The values, of our results are higher than those described by PETERSON *et al.* (8) if they were related to protein concentration. The values obtained by FRYE *et al.* (3) were 3.78 binding sites for mol of bovine albumin; these values from albumin are difficult to compare with the serum ones as there are many types of proteins in serum and this paper considers the total proteins as a whole in order to have a functional approximation.

Resumen

Se determinan las fracciones de calcio, magnesio y estroncio unidas a proteínas de suero humano, así como las libres, en un amplio rango de concentraciones de cada elemento, por una técnica de diálisis. A partir de las curvas de asociación se determinan sus constantes de asociación y su máxima capacidad de unión entre cada uno de los tres elementos por separado y las proteínas de sueros normales, eliminando otros acomplejantes aniónicos del suero. Las curvas son paralelas para los tres elementos, pudiendo ser divididas en tres

partes, correspondiendo la primera a una subfracción fuertemente unida; la segunda, a diferentes puntos con la misma constante de asociación y la tercera a una pendiente de saturación, la cual se alcanza con concentraciones de alcalino-térreo 9 veces superiores a las fisiológicas. El número de puntos de unión es menor para el Sr que para el Ca y el Mg, mientras que la fuerza de la asociación es mayor para el Sr seguida por la del Ca, siendo la más débil la del Mg.

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